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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1.	Your reference	CDK2066
2.	Patent application number *(The Patent Office will fill in this part)*	**0230095.2** 24 DEC 2002
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	RHODIA CONSUMER SPECIALTIES LIMITED Oak House Reeds Crescent Watford Hertfordshire WD24 4QP.
	Patents ADP number *(if you know it)*	848679 7001
	If the applicant is a corporate body, give the country/state of its incorporation	
4.	Title of the invention	PHOSPHORODIAMIDITE
5.	Name of your agent *(if you have one)*	Barker Brettell
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	138 Hagley Road Edgbaston Birmingham B16 9PW
	Patents ADP number *(if you know it)*	7442494002
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country / Priority application number *(if you know it)* / Date of Filing *(day/month/year)*
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application / Date of filing *(day/month/year)*
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request *(Answer 'Yes' if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body. See note (d))*	YES

Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	-
Description	5 + 5
Claim*(s)*	2 + 2
Abstract	-
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10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination *(Patents Form 9/77)*	1
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature Barker Brettell

Barker Brettell

Date 23 December 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Colin D. Kinton Tel: 0121 456 1364

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PHOSPHORODIAMIDITE

The present invention relates to an improved method for the production of phosphorodiamidites, phosphorodiamidites produced by way of such a method and the use of such phosphorodiamidites.

Production of phosphorodiamidites has become increasingly important in the biotechnology industry. Phosphorodiamidites are used as intermediates in the manufacture of novel anti-neoplastic agents.

To be suitable for use in such industries phosphorodiamidites must be of high purity. Such phosphorodiamidites must also contain low levels of bis-(2-cyanoethyl) phosphorodiamidite (the 'diester').

This impurity is known to be a significant by product in the synthesis of 2-cyanoethyl tetraisopropylphosphorodiamidite, a commercially important intermediate in the synthesis of oligonucleotides.

As phosphorodiamidites are very air sensitive and thermally unstable, their purification is, at present, complex and expensive. Hitherto, known processes of extraction and purification of phosphorodiamidites often involve multi-stage synthetic procedures which demand the chemical isolation of intermediate materials and require extensive purification procedures prior to the isolation of high purity phosphorodiamidite products.

The present invention aims to ameliorate the aforementioned disadvantages of phosphorodiamidite production.

Accordingly, the present invention provides a method of phosphorodiamidite production which method comprises the steps of

reacting a phosphorus trihalide with a dialkyl amine in a polar solvent to form an intermediate compound and subsequently reacting the intermediate compound with a hydroxyalkyl compound and a dialkyl amine, in the presence of a non-polar co-solvent.

Following filtration to remove the solid by-product, the two solvents form separate layers. This is advantageous as the upper, non-polar solvent, layer contains the high-purity phosphorodiamidite product. The lower, polar solvent, layer contains impure product contaminated with diester and other unwanted by-products. The upper layer is then subjected to vacuum-stripping to remove the solvent, leaving the desired product with greater than 96% purity and containing less than 1% of the diester impurity. The yield of the product can further be increased by optionally rewashing the polar solvent layer with a further quantity of non-polar solvent, to give non-polar solvent solution containing pure product, from which can then be isolated high-purity phosphorodiamidite.

Advantageously, impure product contaminated with diester and other impurities which would otherwise be unsuitable for commercial use can be extracted and purified by use of the solvent purification procedure. Phosphorodiamidite products are preferentially soluble in the non-polar co-solvent whereas the diester and other unwanted polar by-products are insoluble and remain in the polar solvent layer.

Preferably, the phosphorus trihalide is phosphorus trichloride. Alternatively, the phosphorus trihalide is phosphorus tribromide.

The dialkyl amine is preferably diisopropylamine. Alternatively the dialkyl amine may be dimethylamine, diethylamine, di-n-propylamine, di-n-butylamine, di-isobutylamine or di-tert-butylamine.

The polar solvent is preferably a nitrile compound, in particular, acetonitrile. Alternatively the polar solvent may be propionitrile or benzonitrile.

The hydroxyalkyl compound is preferably hydroxypropionitrile. Alternatively the hydroxyalkyl compound may be methanol, tert-butyl alcohol or other suitable hydroxyalkyl compounds which are known to be suitable for the manufacature of phosphorodiamidites.

The alkane co-solvent is preferably heptane or hexane. Other suitable C_5 to C_9 aliphatic hydrocarbons include pentane. Suitable alicyclic hydrocarbons include, for example, cyclohexane.

The ratio of polar solvent to non-polar solvent is suitably around 1:1. The method according to the invention provides a phosphorodiamidite compound according to Formula I:

$$(R_2\,N)_2\text{-P-O}(CH_2)_n\text{-CN} \qquad \text{(I)}$$

wherein R is a C_1 to C_4 alkyl, hydroxyalkyl or oxyalkyl group; and n is a whole number of from 1 to 4.

The compound according to formula I is preferably 2-cyanoethyl tetraisopropyl phosphorodiamidite wherein R is isopropyl, and n = 2.

The present invention also provides the use of a compound of formula I in the synthesis of oligonucleotides.

The present invention will now be illustrated, merely by way of example, as follows:

Example 1

Manufacture of 2-cyanoethyl tetraisopropyl phosphorodiamidite using hexane co-solvent

27.5g of phosphorus trichloride at ambient temperature was added to a stirred mixture of acetonitrile (200g) and diisopropylamine (121g) over 1 hour. 200g of hexane is then added followed by 14g of hydroxypropionitrile at ambient temperature over 30 minutes. The reaction mixture is then stirred for 1 hour and is then filtered to remove the solid by-product. The upper hexane layer of the filtered reaction mixture is separated and subjected to vacuum stripping to remove the hexane solvent. This leaves 20g of 2-cyanoethyl tetraisopropylphosphorodiamidite which has a purity of 96.9% when analysed by ^{31}P-NMR. The lower acetonitrile layer is stirred with a further 200g of hexane for 2 hours. The upper hexane layer from this re-extraction contains product of 98% purity when assayed by ^{31}P-NMR. Following vacuum stripping a further 11g of high purity 2-cyanoethyl tetraisopropylphosphorodiamidite is isolated.

Example 2

Manufacture of 2-cyanoethyl tetraisopropylphosphorodiamidite using heptane co-solvent

27.5g of phosphorus trichloride was added to a stirred mixture of 200g of acetonitrile and 121g of diisopropylamine at ambient temperature. 200g of heptane was then added to this mixture followed by 14.3g of hydroxypropionitrile at ambient temperature over 30 minutes. The reaction mixture was then stirred for an hour and was then filtered to remove the solid by-product. The upper heptane layer was then separated

and subjected to vacuum stripping to remove the heptane solvent leaving 22g of 2-cyanoethyl tetraisopropylphosphorodiamidite which had a purity of 96.7% when assayed by ^{31}P-NMR.

Example 3

Purification of low purity 2-cyanoethyl tetraisopropylphosphorodiamidite

60g of low purity 2-cyanoethyl tetraisopropylphosphorodiamidite (92% purity when assayed by ^{31}P-NMR containing 1.3% diester) was added to a mixture of 200g acetonitrile and 200g of heptane after stirring for ten minutes the upper heptane layer was separated and the lower acetonitrile layer stirred with a further 200g of heptane for a further 10 minutes. The second heptane fraction was then separated and the two heptane fraction subsequently combined and subjected to vacuum stripping to remove heptane solvent. 30g of 2-cyanoethyl tetraisopropylphosphorodiamidite was obtained at a purity of 98.3% when assayed by ^{31}P-NMR. This extracted phosphorodiamidite compound contained less than 0.1% of the diester impurity.

CLAIMS

1. A method of phosphorodiamidite production which method comprises the steps of reacting a phosphorus trihalide with a dialkyl amine in a polar solvent to form an intermediate compound and subsequently reacting the intermediate copound with a hydroxyalkyl compound and a dialkyl amine, in the presence of a non-polar co-solvent.

2. A method as claimed in Claim 1 in which the phosphorus trihalide is phosphorus trichloride.

3. A method as claimed in Claim 1 in which the phosphorus trihalide is phosphorus tribromide..

4. A method according to any one of Claims 1 to 3 in which the dialkyl amine is diisopropylamine.

5. A method as claimed in any one of Claims 1 to 3 in which the dialkyl amine is selected from the group consisting of dimethylamine, diethylamine, di-n-propylamine, di-n-butylamine, di-isobutylamine or di-tert-butylamine.

6. A method as claimed in any one of the preceding claims in which the polar solvent is a nitrile compound.

7. A method as claimed in Claim 6 in which the nitrile compound is acetonitrile.

8. A method as claimed in Claim 6 in which the polar solvent is propionitrile or benzonitrile.

9. A method as claimed in any one of the preceding claims in which the hydroxyalkyl compound is hydroxypropionitrile.

10. A method as claimed in any one of Claims 1 to 8 in which the hydroxyalkyl compound is methanol or tert-butyl alcohol.

11. A method as claimed in any one of Claims 1 to 10 in which the alkane co-solvent is a C_5 to C_9 aliphatic hydrocarbon.

12. A method as claimed in any one of Claims 1 to 10 in which the alkane co-solvent is an alicyclic hydrocarbon.

13. A method according to any one of the preceding claims in which the ratio of polar solvent to non-polar solvent is 1:1.

14. A phosphorodiamidite compound produced by the method of any one of Claims 1 to 13 and having the General Formula (I):

$$(R_2\,N)_2\text{-}P\text{-}O(CH_2)_n\text{-}CN \qquad (I)$$

wherein R is a C_1 to C_4 alkyl, hydroxyalkyl or oxyalkyl group; and n is a whole number of from 1 to 4.

15. A compound according to Claim 14 which is 2-cyanoethyl tetraisopropyl phosphorodiamidite.

16. The use of a compound as claimed in Claim 14 or Claim 15 in the synthesis of oligonucleotides.

17. A phosphorodiamidite compound, substantially as hereinbefore described with reference to the Examples.